bio-protocol Preprint

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Imaging mass cytometry staining - fresh frozen human brain tissue sections

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An abbreviated version of this protocol was published in eLIFE in Aug 2019

Multiplexed imaging of immune cells in staged multiple sclerosis lesions by mass cytometry

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Detailed protocol

Note: Use filtered tips for all steps to avoid cross-contamination with metals

Prior to the staining:

1. Human brain tissue blocks are embedded in Tissue Tek Optimum Cutting Temperature (OCT) medium and 10µm thick sections are cut using a cryostat.

2. The frozen tissue sections are mounted on Superfrost Plus glass slides (Knittel Glass) and stored at -80˚C until they are stained.

Day 1

1. Remove the slide from -80˚C and allow to warm up to room temperature in a closed chamber to avoid condensation

2. Using a wash bottle, rinse the tissue section with Ultrapure sterile ddH_2O to remove the OCT

3. Tap slide to discard volume. Dry the slide by dabbing the area around the tissue section with a Kim Wipe

4. Use a hydrophobic pen to draw a barrier around the tissue section and allow it to dry. Take care to avoid any leakage of the hydrophobic solution onto the tissue and to avoid breaks along the barrier which would not contain the staining solutions in the subsequent steps

5. Place the slide inside a humidified chamber and incubate the tissue section in 0.05% PBS-Tween for 20 minutes to re-hydrate

6. Tap slide to discard the PBS-Tween. Dry the slide by dabbing the area around the tissue section with a Kim Wipe

7. Place the slide inside a humidified chamber and incubate the tissue section in 2-3 drops (sufficient volume to cover the section) of streptavidin blocking solution (Vector, SP-2002). Incubate for 15 minutes at room temperature

8. Tap slide to discard the streptavidin blocking solution. Transfer the slide to a coplin jar and rinse the tissue section in PBS-Tween (0.05%) for 5 mins at room temperature on a slow-moving shaker

9. Place the slide inside a humidified chamber and incubate the tissue section in 2-3 drops (sufficient volume to cover the section) of biotin blocking solution (Vector, SP-2002). Incubate for 15 minutes at room temperature

10. Tap slide to discard the streptavidin blocking solution. Transfer the slide to a coplin jar and rinse the tissue section in PBS-Tween (0.05%) for 5 mins at room temperature on a slow-moving shaker

11. Place the sections inside a humidified chamber and incubate in 10% normal goat serum solution in PBS for 1 hour at room temperature

12. Tap slide to discard the normal goat serum. Dry the slide by dabbing the area around the tissue section with a Kim Wipe

13. Place the sections inside a humidified chamber and incubate in SuperBlock (PBS) Blocking Buffer(ThermoFisher, 37518) for 45 minutes at room temperature

14. Make up the cocktail of metal-conjugated antibodies (see Table of primary antibodies) in 0.5% BSA in PBS.

15. Tap slide to discard the superblock buffer. Dry the slide by dabbing the area around the tissue section with a Kim Wipe

16. Place the sections inside a humidified chamber and incubate in the cocktail of metal-conjugated antibodies overnight at 4˚C

Day 2

17. Tap slide to discard the antibody cocktail. Transfer the slide to a coplin jar and rinse the tissue section in PBS-0.05% Tween 2X for 8 minutes at room temperature on a slow-moving shaker

18. Using a wash bottle, further rinse the tissue section with PBS-0.05% Tween

19. Using a wash bottle, further rinse the tissue section with PBS

20. Place the slide inside a humidified chamber and stain the tissue section with Ir-intercalator diluted 1/2000 in 0.5% BSA in PBS for 30 minutes at room temperature

21. Tap slide to discard the Ir-intercalator. Transfer the slide to a coplin jar and quickly rinse the tissue section in Ultrapure sterile ddH_2O for 5 seconds at room temperature on a slow-moving shaker. Do not wash for too long or the signal will dim

22. Tap slide to discard the water and let the section air dry for 20 minutes

23. Place the dried stained slide inside a slide box. Place the slide box inside a vacuum-sealed plastic bag with desiccant and store at room temperature until it is imaged.

Table of primary antibodies

Target	Source	Identifiers	Dilution
Anti-Nucleic Acid-Ir191/Ir193	Fluidigm	Cat#:201192A RRID: AB_2810850	1/3000
Anti-Proteolipid Protein-141Pr (Mouse monoclonal)	Bio-Rad	Cat#: MCA839G RRID:AB_2237198	1/25
Anti-human CD38-167Er (Mouse monoclonal)	Fluidigm	Cat#:3167001B RRID: AB_2802110	1/2000
Anti-human CD45-154Sm (Mouse monoclonal)	Fluidigm	Cat#: 3154001B RRID:AB_2810854	1/2000
Anti-human CD68-159Tb (Mouse monoclonal)	Fluidigm	Cat#: 3159035D RRID:AB_2810859	1/100
Anti-human HLA-147Sm (Mouse monoclonal)	Fluidigm	Cat#: Ab55152 RRID: AB_944199	1/100
Anti-human TMEM119-155Gd (Rabbit polyclonal)	Sigma-Aldrich	Cat#: HPA051870 RRID: AB_2681645	1/50
Anti-human CD3-170Er (Mouse monoclonal	Fluidigm	Cat#: 3170001 RRID: AB_2661807	1/100
Anti-human CD4-176Yb (Mouse monoclonal)	BioLegend	Cat#:344602 RRID: AB_1937277	1/100
Anti-human CD8a-162Dy (Mouse monoclonal)	Fluidigm	Cat#: 3162015B RRID:AB_2661802	1/100
Anti-human Granzyme B-171Yb (Mouse	ThermoFisher Scientific	Cat#: MA1-80734 RRID:AB_931084	1/25

monoclonal)			
Anti-human IgKappa-160Gd (Mouse monoclonal)	Fluidigm	Cat#:3160005B RRID:AB_2810855	1/3000
Anti-human IgLambda-151-Eu (Mouse monoclonal)	Fluidigm	Cat#: 3151004B RRID:AB_2810853	1/3000
Anti-human IgM-172Yb (Mouse monoclonal)	Fluidigm	Cat#: 3172004B RRID:AB_2810858	1/500
Anti-human Collagen Type I-169Tm (Goat polyclonal	Fluidigm	Cat#: 3169023D RRID:AB_2810857	1/4000
Anti-human CD31-145Nd (Mouse polyclonal)	LSBio	Cat#: LS-C390863 RRID:AB_2810860	1/100
Anti-human NFAT1-143Nd (Rabbit monoclonal)	Fluidigm	Cat#: 3143023A RRID:AB_2810851	1/50
Anti-human Ki67-168Er (Mouse monoclonal)	Fluidigm	Cat#: 3168001B RRID:AB_2810856	1/100

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ramaglia, V. and Sheikh-Mohamed, S. (2020). Imaging mass cytometry staining - fresh frozen human brain tissue sections. Bio-protocol Preprint. bio-protocol.org/prep266.
2. Ramaglia, V., Sheikh-Mohamed, S., Legg, K., Park, C., Rojas, O. L., Zandee, S., Fu, F., Ornatsky, O., Swanson, E. C., Pitt, D., Prat, A., McKee, T. D. and Gommerman, J. L.(2019). Multiplexed imaging of immune cells in staged multiple sclerosis lesions by mass cytometry. eLIFE. DOI: 10.7554/eLife.48051